THE USE OF EXCISED SHOOTS IN LINSEED INVESTIGATIONS.

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(Plate xiv.)

[Read 28th November, 1951.]

Synopsis.

Investigations dealing with physiologic specialization in *Melampsora lini* (Pers.) Lév. and the genetics of resistance in crosses between varieties of *Linum usitatissimum* L. have been in progress at Sydney University since 1940. Techniques used by other workers were found to have certain disadvantages. The excised shoot technique has recently been developed to overcome these problems. It involves the excision of shoots above the cotyledons, and subsequent growth in tap water or nutrient solution. The advantages of the method are discussed and further developments indicated.

Introduction.

During 1948 investigations were commenced at Sydney University with particular reference to the inheritance of resistance to *Melampsora lini* (Pers.) Lév. The techniques adopted were those used by previous investigators (Waterhouse and Watson, 1941, 1943; Baker, 1945; Charles, 1947) at Sydney University, and patterned on U.S.A. work (Flor, 1947). Flor, of U.S.A., in his genetical investigations grew and tested his material in four and a half inch pots. He used the tip inoculation method, removing infected leaves as soon as the reactions became distinct and inoculating the same plants with new races of rust at 7–8-day intervals. Here this method was soon found to have distinct limitations.

It was frequently necessary to allow infected leaves to remain on the plant for some time after sporulation in order to distinguish between susceptible and certain mesothetic reactions. Spores liberated at this time were a serious source of contamination because these plants usually had to be inoculated with other races of rust. The most susceptible segregates usually had to be removed. Others, greatly weakened by the rust attack, often succumbed to root rot or a later rust infection. Even when infected leaves were removed, it was impossible to control the rust spread to the cotyledons and stem. In order to reduce contamination the infected shoot was cut back and fresh growth from the base inoculated with the second race. This control measure was only moderately successful, since prolonged growth of the plants in the glass house and successive rust inoculations made the plants particularly liable to root rot.

Development of the Excised Shoot Technique.

During 1948 it was noticed that linseed seedling tips, cut or broken off and left lying on a free water surface, continued to grow and soon developed a strong root system. Shoots of several varieties placed in tap water gave normal reactions when inoculated with different races of rust. Arnon's nutrient solution (Arnon, 1938) was subsequently used and a more vigorous growth obtained. The value of these observations was apparent, and after preliminary trials a new technique for linseed investigations has been developed.

Equipment and Method.

The equipment consists of a Pyrex dish approximately 12″ × 8″ × 2″ of about two litres capacity. Fitting into this is an aluminium grid made of two sheets clamped about half an inch apart. The bottom sheet, of slightly smaller dimensions than the dish, fits into it and is suspended in position by the top sheet, which slightly overlaps the edge of the dish. One hundred and forty ¼″ holes arranged in 14 rows of 10 holes each are bored through both sheets (Plate xiv, fig. A). Although suspension of the grid in tap water or nutrient solution did not visibly affect the plant growth or rust reaction, it was lacquered as a precautionary measure. Thanks are due to the Food Preservation and Transport Division of the C.S.I.R.O. for this lacquering.

Seedlings to be tested with different races of rust are sown in four-inch pots. The seedlings are identified by the pot number and by loops of bell wire of different shapes and colour. When the seedling is about three inches high the growing shoot is excised about half an inch above the cotyledons and placed in a hole in the grid corresponding to its number in the series. The shoot may be inoculated with rust immediately, but if it shows signs of wilting may be stood over for a short period to recover. As the rust infection develops, the shoot forms a callus, often very large (Plate xiv, fig. D), and if rust infection is not severe it roots within three weeks (Plate xiv, fig. E). The rooted shoots may be transplanted and grown to maturity: quite sturdy plants have been obtained from the weakest specimens. Alternatively the shoots may be left to set seed *in situ* if it is desired to determine the flower colour or obtain only a small quantity of seed.

While the first shoot is being tested, the parent plant, kept out of range of infection, will throw out at least two basal shoots, which are available for further use. When ten plants have been grown in a four-inch pot, five shoots per plant have been obtained without difficulty, and this number may easily be increased by reducing the number of plants per pot or by cutting back rooted shoots and using them as a source of further shoots. A very large number may be obtained from plants growing in the field. The tendency of the main stem of vigorously growing spaced plants in the field to develop a heavy crop of buds from the leaf axils may be used to advantage. One hundred such shoots have often been counted on the one stem.

Modified Techniques.

The first has been developed for the race differentiation studies.

Several excised shoots of each of the 20 differential varieties are bound in bundles with a single twist of bell wire to weight them, and then placed in 2″ × 1″ glass tubes arranged in two rows of 10 in a wooden stand 3½″ × 1¼″ × 16″. The shoots in position are inoculated and incubated in the usual way. Clearly a great saving of space is effected (Plate xiv, fig. C).

Quarter-pint cream bottles and other larger glass containers have also been used (Plate xiv, fig. B). It is not necessary to water the shoots so frequently, since they fall with the level of the water in the container. These containers may be capped and used as incubation chambers by temporarily lowering the water surface till the shoots fall below the level of the mouth. After incubation the water level may be raised and the shoots floated back above the mouth.

Sydney tap water has been quite adequate to maintain growth and obtain normal rust reactions. Growth is not so rapid and succulent as in nutrient solution, but this is an advantage if the shoots have to be held back for any period prior to transplanting.

Root and Callus Formation.

Preliminary studies indicate that the rate and vigour of rooting of the excised shoot are not markedly determined by the size of the shoot nor by the meristematic activity of the growing point. Shoots from which the growing points have been removed appear to root as readily as normally excised shoots, so that a single stem may be cut into several lengths and each length rooted. By the time such cuttings have rooted, axillary buds have developed at the top and normal vegetative growth is resumed. Excised shoots from which the growing points have been removed and left lying horizontally on the surface of the water frequently take up water and develop a distinct callus at the lower cut surface. A vigorous callus forms even when such a shoot is inverted with the upper end under water and the cut surface exposed to the air. A large branch of the inflorescence of one plant, half broken away from the main stem, was held in place with bell wire. It continued to grow normally and within a fortnight the injured surface had developed a strong callus.

Associated with this vigorous callus-forming capacity is the tendency, observed in a recent summer sowing of young seedlings, to develop small suppressed green hypocotyledonary buds. The bulk of these buds was observed at soil level, but many were noticed slightly higher up. Their development was probably stimulated by the light friction of the hypocotyledonary epidermis against the sandy soil particles in windy weather.

Advantages of the Excised Shoot Technique.

Since excised shoots are used problems of rust contamination are eliminated. Bench space requirements are reduced by more than 50%. The equipment is more easily handled and is cleaner than where pots are used, and the nutrient level can be kept constant from one test to the next. The use of young shoots eliminates any risk of rust reactions varying with increasing age. Root rotting organisms like *Pythium* and *Fusarium* have not yet affected growth of the shoots in solution.

The prolific shooting capacity of linseed makes it possible to test the one plant with a great number of races simultaneously or consecutively. Uninfected shoots may be kept in reserve against the loss of the parent plant or transplanted into the field for seed increase.

It is useful in plant breeding work, where it may sometimes be necessary to test a plant with a particular pathogen without exposing the parent plant to the disease or introducing the disease to the field. A rapid increase of valuable plants is possible. Thus a single plant has been increased a hundredfold in the last four months. Since each new plant can now be used for further increase, this number could be quadrupled in a fortnight. This should help to offset the relatively low seed yield obtained from fibre types. It may also be possible to maintain a particular plant or variety vegetatively from one season to the next and then increase it clonally. This will overcome problems of cross pollination.

Problems Encountered.

A few set-backs have been experienced in the course of this work.

The shoots succumbed rapidly to a bacterial invasion in hot weather, when overcrowded and grown under conditions of low light intensity. Chlorotic shoots suffered more severely than normal shoots, and unrooted more than rooted shoots. No trouble has been experienced recently, since every third row in the aluminium grid has been left empty to allow for better light conditions and circulation of air. It is not yet certain whether excised shoots will live through the summer without controlled-temperature facilities.

Two or three weeks after they have been placed in Sydney tap water containing Arnon's nutrient solution minus the trace elements, excised shoots pass through a stage when chlorosis of the new growth occurs. This does not affect the rust reactions shown by the leaves that were inoculated earlier. Later, with the addition of tap water to make up for loss caused by transpiration and evaporation, the chlorotic condition disappears. The chlorosis is probably due to a temporary trace element deficiency accentuated during rapid growth, but remedied by subsequent addition of tap water. Initial experiments suggest an iron deficiency. The addition of rusty nails to the solution has given adequate control.

Conclusion.

The technique has now been developed beyond the experimental stage and has become the basis of all glass-house studies at Sydney University. One hundred of the aluminium grids and dishes are in use, together with the equipment for the modified techniques.

Several interesting lines of work which are worthy of investigation are opening up, as for example, nutritional studies, their effects on rust reaction prior to and after rooting, studies on rooting physiology, and the relation of root development to general plant growth. Heterosis studies based on terminal growth rates of the shoot are made easy since lateral bud development is normally suppressed.

Other avenues are opening up as the work proceeds.

Acknowledgements.

Grateful acknowledgement is made to Meggitt Ltd., whose grant of financial aid has made this work possible.

I also wish to express my special indebtedness to Professor W. L. Waterhouse for his constant encouragement and advice.

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EXPLANATION OF PLATE XIV.

A : Grid with excised shoots in position and lifted out of pyrex dish to show heavy root development after about four weeks. × 1/3.

B : Cream jar with shoots floated above mouth of jar after incubation. × 5/9.

C : A set of the rust differential varieties in which the excised shoots are used in short specimen tubes placed in position in a frame. × 3/16.

D : Basal stems of excised shoots, after about five weeks, with roots removed to show extreme cases of callus formation. × 1.

E : Extensive root development shown by an excised shoot after about four weeks. Top growth removed for photographic purposes. × 1.